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Effect of pyrethroids, permethrin and fenvalarate, on the oxidative stress of *Helicoverpa armigera*

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Abstract

The cotton bollworm, *Helicoverpa armigera* is a polyphagous pest of several crops in Asia, Africa, and the Mediterranean Europe. Pyrethroid insecticides are used to control noxious insects in agriculture, forestry, households and horticulture. Therefore, the effect of permethrin and fenvalarate was evaluated on the oxidative stress in *H. armigera*. Lipid peroxidation, H_2O_2 content, and lactate dehydrogenase (LDH) activity increased and glutathione reductase (GR) activity decreased in a time- and dose-dependent manner in insecticide-fed larvae. However, catalase activity was not affected in insecticide-fed larvae. Larval growth decreased by ~ 64 and 70% in larvae fed on diets with sub-lethal doses of permethrin and fenvalarate. The results suggested that both the insecticides induced lipid peroxidation, H_2O_2, LDH leak, and altered antioxidant enzymes leading to oxidative stress in cells, which contribute to deleterious effects of these insecticides on the growth of *H. armigera* larvae, along with their neurotoxic effects.

Keywords: *Helicoverpa armigera,* permethrin, fenvalarate, oxidative stress, antioxidant enzymes.

INTRODUCTION

Cotton bollworm/legume pod borer, *Helicoverpa armigera* (Noctuidae: Lepidoptera), is one of the major constraints to crop production in Asia, Africa, Australia and the Mediterranean Europe. It is a polyphagous pest and has been reported to attack more than 200 different species of plants including cotton, pigeonpea, groundnut sorghum, maize, chickpea, vegetables, fruit and forest trees (Sharma *et al* 2003). Plants are continuously exposed to the challenge of a variety of herbivores. Lepidopteran larvae are essential 'eating machines', and grow much faster than the young mammals and birds with high metabolic processes (Chamberlin 2004). Insecticides exhibit a high level of pest control ability combined with a relatively low degree of environmental toxicity; hence, they are used widely around the world in agriculture and in households. Almost all classes of insecticides are neurotoxic, there are number of reports stating the toxic effects of insecticides on insects as well as on non-target groups with different target sites. Studies on the effects of the pesticides have mainly been carried out *in vivo* in rodents (Piña-Guzmán *et al* 2005), Pisces (Li and Zhang 2002) and in few mammals such as pig (Campagna *et al* 2002). However, *in vivo* effect of insecticides in insect system is less available, thus we evaluated the *in vivo* effect of pyrethroids in *H. armigera*. Pyrethroids are toxic to the central nervous system of both insects and mammals (Husain *et al* 1996). Alteration in sodium channel kinetics is the principal molecular mode of action of pyrethroids (Tatebayashi and Narahashi 1994). Inhibitory effects have also been observed for Ca^{2+} channels (Kadous *et al* 1994) and ATPases (Reddy *et al* 1991).

Induction of oxidative stress is one of the main mechanisms of the action of many insecticides. Some organophosphates and carbamates have been found to induce mitochondrial dysfunction and oxidative stress in rat (Lukaszewicz-Hussain and Moniuszko-Jakoniuk 2004, Kamboj *et al* 2008). Pesticide exposure has induced superoxide, H_2O_2 and altered the level of antioxidant enzymes in mice (Olgun and Misra 2006). Exposure of rats to a single dose of the pyrethroids, cypermethrin (25 µg kg−1) and fenvalerate (4.5µg kg−1), lowered the activities of the antioxidant enzymes superoxide dismutase and catalase, resulting in both lipid peroxidation and decreased levels of reduced glutathione (GSH) in erythrocytes (Kale *et al*., 1999). Cypermethrin, a pyrethroid, induced the oxidative metabolism in fish (Ghosh 1989). The present studies were thus aimed to evaluate the effect of permethrin and fenvalarate, on the oxidative stress, which could affect the growth and development of *H. armigera*.

MATERIALS AND METHODS

Chemicals

NADH, bovine serum albumin, ADP were purchased from Sigma Aldrich (Mumbai, India). Sucrose was purchased from Qualigens (Mumbai, India). Permethrin (99%) and fenvalarate (99.4%) from Dow Agrosciences were gifted from Dr Sharom, Department of Environmental Biology, University of Guelph (Guelph, ON, Canada), while the other chemicals used were of analytical grade.

Insects

Larvae of *H. armigera* were obtained from the insect rearing laboratory, International Crops Research Institute for the Semi-Arid

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Tropics (ICRISAT), Patancheru, Andhra Pradesh, India. The larvae were reared on a chickpea based semi-synthetic diet under laboratory conditions at 27 ± 1 ˚C, 65 ± 5% RH, and 12 h photoperiod (Armes *et al* 1992).

Lipid peroxidation

Fourth-instar larvae were fed with different concentrations of permethrin and fenvalarate (0 - 100 µM), and lipid peroxidation was measured by quantifying malondialdehyde (MDA) level in larval homogenates on the basis of reaction with thiobarbituric acid to form a pink colored complex. MDA produced was measured at 532 nm, and the nonspecific absorbance was subtracted by measuring the absorbance at 600 nm. Lipid peroxidation was calculated using 1.56 X 10^5 as extinction coefficient and expressed as µmol of MDA/mg of protein extract (Poovala *et al* 1999). Protein concentration was estimated by Lowry's method (Lowry *et al* 1951).

Lactate dehydrogenase leakage

Fourth-instar larvae were fed with different concentrations of permethrin and fenvalarate (0-100µM), and lactate dehydrogenase (LDH) activity was determined in larval homogenates by measuring decrease in NADH content at 340 nm by using UV spectrophotometer (Hitachi, U-2900) and the enzyme activity was expressed as mmoles/min/mg protein (Poovala *et al* 1999).

Measurement of H_2O_2 content

Fourth-instar larvae were fed with different concentrations of permethrin and fenvalarate (0 - 100 µM), and H_2O_2 content was estimated in larval homogenates according to Noreen and Ashraf (2009), and expressed as µmoles of H_2O_2/mg protein.

Assay of antioxidant enzymes

Antioxidant enzymes were assayed in insecticide-fed larvae spectrophotometrically. Catalase activity was determined by the kinetic assay adapted from Olgun and Misra (2006), in which the disappearance of peroxide is monitored spectrophotmetrically at 240 nm. One unit of catalase is equivalent to µmol of H_2O_2 decomposed per minute per mg of protein using the extinction coefficient of 43.6 M^{-1} cm^{-1}. Glutathione reductase (GR) was determined with 1 ml reaction mixture, containing 1 ml 50 mM phosphate buffer, pH 7.2, 1 mM EDTA, 0.05% bovine serum albumin, 10 mM oxidized glutathione, and 10 mM NADPH. The rate of change of absorbance was measured at 340 nm. One unit of enzyme activity was expressed as µmol of NADPH oxidized per minute per mg of protein (Olgun and Misra, 2006).

Bioassay of insecticides

Permethrin and fenvalarate were incorporated into the artificial diet at different concentrations (0 to 100 µM). Third-instar larvae were released into the insecticide containing diet, their initial weights were measured before they were released into the diet. There were three replications for each treatment in completely randomized design, 10 larvae in each replication. The larval weights were recorded 5 days after initiating the experiment.

Statistical analysis

Data were subjected to one way analysis of variance (ANOVA) to judge the significance of difference between the treatments by using F-test, while the significance of differences between the treatment means was judged by least significant difference (LSD) at $p < 0.05$.

RESULTS

Lipid peroxidation, H_2O_2 content and lactate dehydrogenase leak

In control larvae, lipid peroxidation, H_2O_2 content, and LDH leak were found to be 0.5 mmoles/min/mg protein, 2.34 µmoles/min/mg protein, 0.116 µmoles/min/mg protein, respectively. Lipid peroxidation (Fig. 1,2), LDH leak (Fig. 3, 4) and H_2O_2 content (Fig. 5,6) were estimated in insecticide fed larvae. There was proportional increase in these components, all the three parameters increased significantly after 18 h of feeding of insecticide containing diet, their concentration reached maximum at 24 h. For dose response, lipid peroxidation, LDH leak and H_2O_2 content were estimated after 24 h, and up to 140%, 201%, 44% and 90%, 183%, 61% increase in these components was observed with 100 µM of permethrin and fenvalarate, respectively. Thus there was a proportional increase in these components in time- and dose-dependent manner in insecticide-fed larvae.

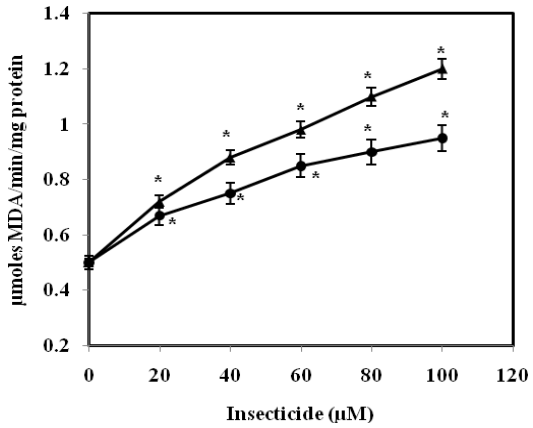

Fig 1. Dose-dependent response for lipid peroxidation in the larvae fed on diet containing permethrin (▲) and fenvalarate (●). The data represents the Mean ± S.D. (n = 3). (Significantly different from control at * $p < 0.05$).

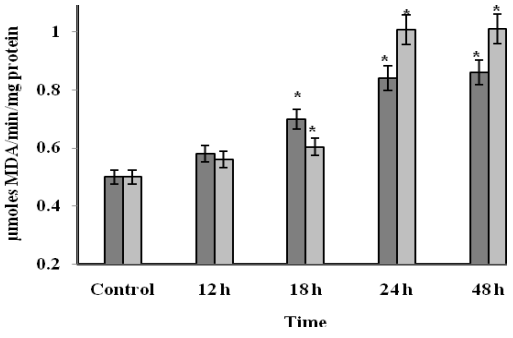

Fig 2. Time-dependent response for lipid peroxidation in the larvae fed on diet containing permethrin ( ) and fenvalarate ( ). The data represents the Mean ± S.D. (n = 3). (Significantly different from control at * $p < 0.05$).

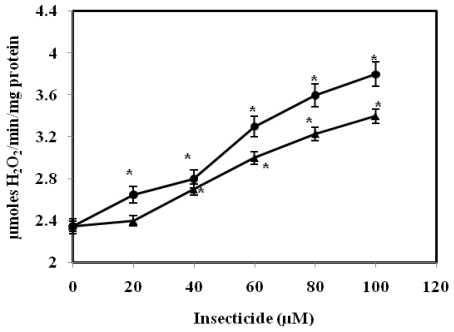

Fig 3. Dose-dependent response for H_2O_2 production in the larvae fed on diet containing permethrin (▲) and fenvalarate (●). The data represents the mean ± S.D. (n = 3). (Significantly different from control at * p < 0.01).

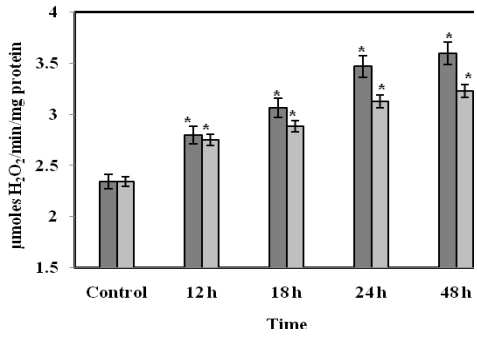

Fig 4. Time-dependent response for H_2O_2 production in the larvae fed on diet containing permethrin ( ) and fenvalarate ( ). The data represents the mean ± S.D. (n = 3). (Significantly different from control at * p < 0.01).

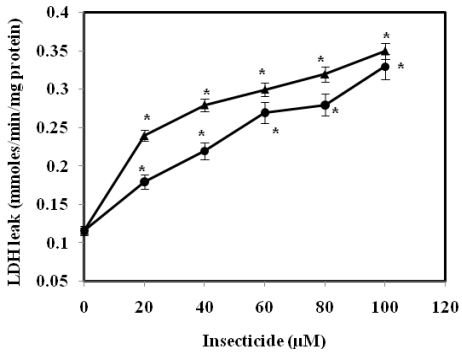

Fig 5. Dose-dependent response for LDH leak in the larvae fed on diet containing permethrin (▲) and fenvalarate (●). The data represents the mean ± S.D. (n = 3). (Significantly different from control at * p < 0.05).

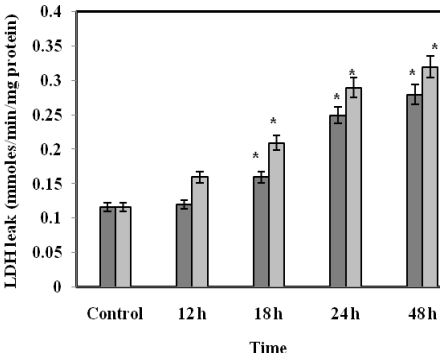

Fig 6. Time-dependent response for LDH leak in the larvae fed on diet containing permethrin ( ) and fenvalarate ( ). The data represents the mean ± S.D. (n = 3). (Significantly different from control at * p < 0.05).

Antioxidant enzymes

In the present studies, the activity of GR was reduced in dose- and time-dependent manner, whereas, catalase was not affected in permethrin- and fenvalarate-fed larvae (Table 1,2).

Table 1. Dose-dependent response of antioxidant enzymes in *H. armigera* fed on diet containing insecticide.

	0 µM	20 µM	40 µM	60 µM	80 µM	100 µM
Permethrin-fed larvae						
Catalase (U/mg)	11.74 ± 1.23	11.73 ± 0.89	11.74 ± 0.37	11.78 ± 0.83	11.82 ± 1.34	12.39 ± 1.34
Glutathione reductase (U/mg)	40.57 ± 2.64	36.51 ± 1.22*	28.39 ± 3.82*	25.31 ± 2.82*	22.32 ± 1.83*	15.01 ± 2.73*
Fenvalarate-fed larvae						
Catalase (U/mg)	11.74 ± 1.23	11.64 ± 0.99	11.34 ± 0.98	12.34 ± 1.34	12.44 ± 1.11	12.84 ± 1.12
Glutathione reductase (U/mg)	40.57 ± 2.64	34.89 ± 2.73*	27.38 ± 2.82*	23.93 ± 1.22*	20.08 ± 1.22*	18.66 ± 2.11*

Values in the Table represents Mean ± S.D. of at least three determinants (significantly different from control at * p < 0.05).

Table 2. Time-dependent response of antioxidant enzymes in *H. armigera* fed on diet containing insecticide.

	Control	12 h	18 h	24 h	48 h
Permethrin-fed larvae					
Catalase (U/mg)	11.74 ± 1.23	11.99 ± 0.85	12.12 ± 0.99	12.39 ± 1.34	12.44 ± 1.11
Glutathione reductase (U/mg)	40.57 ± 2.64	36.49 ± 3.28	22.63 ± 2.73*	15.01 ± 2.73*	16.73 ± 1.22*
Fenvalarate-fed larvae					
Catalase (U/mg)	11.74 ± 1.23	11.82 ± 1.87	12.45 ± 0.54	12.84 ± 1.12	12.92 ± 1.34
Glutathione reductase (U/mg)	40.57 ± 2.64	34.96 ± 2.87*	29.43 ± 1.82*	18.66 ± 2.11*	19.45 ± 2.63*

Values in the Table represents Mean ± S.D. of at least three determinants (significantly different from control at * p < 0.05).

Bioassay

Both the insecticides inhibited the larval growth in a dose-dependent manner. At 100 µM, 64 and 70% inhibition in larval growth was observed in permethrin- and fenvalarate-fed larvae, respectively (Fig. 7).

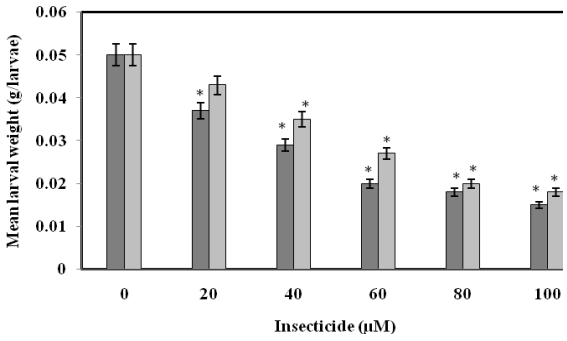

Fig 7. Bioassays for permethrin and fenvalarate. Neonates were fed on artificial diet containing varying concentrations of permethrin ( ) and fenvalarate ( ). The data represents the Mean ± S.D. (n = 3) (Significantly different from control at * p < 0.05).

Discussion

In the present studies, effect of permethrin and fenvalarate was evaluated on the oxidative stress in *H. armigera*. Chemical toxic pollutants are important sources of ROS in biological systems (Bacchetta 2011). A time- and dose-dependent increase in lipid peroxidation, H_2O_2 content and LDH leak was recorded in

insecticide-fed larvae. There was a little increase in oxidative stress markers after 12 h of feeding on diets containing the insecticides, but a significant increase was recorded after 18 h, reaching the maximum level at 24 h. Inhibition of electron transport chain at any site could lead to generation of H_2O_2 and ROS, which in turn peroxidise membrane lipids, as evidenced by high levels of MDA in insecticide-fed larvae. These findings are similar to earlier reports, wherein, toxicity of many xenobiotics, including pesticides has been found to be associated with the generation of ROS (Sohn *et al*. 2004). The LDH activity is the most sensitive parameter for evaluation of tissue damage and toxicity. Significant increase in LDH activity in insecticide-fed larvae indicated the higher rates of glycolysis, indicating that aerobic oxidation was adversely affected in insecticide-fed larvae, as confirmed by inhibition in oxygen uptake *in vivo*. Elevated levels of LDH activity have been associated with inhibition of aerobic oxidation in pesticide exposed fish (Ghosh 1989). Bidrin, an organophosphate insecticide, induced lipid peroxidation, H_2O_2 and LDH levels in cultured renal tubular cells (Poovala 1999), while carbofuran also induced lipid peroxidation in rat brain (Kamboj 2008).

Pesticides are known to alter the level of antioxidant enzymes. In insecticide-fed *H. armigera* larvae, catalase activity was unaffected whereas glutathione reductase was inhibited in a dose- and time-dependent manner. Similar observations have earlier been made by which Olgun and Misra (2006). However, lindane, an organochlorine insecticide, reduced the activity of liver catalase, but did not affect glutathione reductase (Janquiera *et al*. 1986). Because the K_m value for the catalysis of H_2O_2 by catalase is in the range of 1.1 M, and the increased levels of H_2O_2 produced during exposure to insecticides never exceeded this level, no change in levels of catalase are not surprising. (Olgun and Misra 2006). Reduction in glutathione reductase levels may be because of direct effects of these insecticides and their metabolites on this enzyme. (Olgun and Misra 2006).Reduction in larval growth in larvae fed on diets with these insecticides may be due to the increase in oxidative stress as evidenced by high levels of lipid peroxidation, H_2O_2 content, and LDH leak, under *in vivo* conditions.

In conclusion, permethrin and fenvalarate exposure induced lipid peroxidation, H_2O_2 content and LDH leak in a time- and dose-dependent manner, and altered the activities of antioxidant enzymes leading to oxidative stress in cells, resulting in deleterious effects on the growth of *H. armigera* larvae, along with the neurotoxic effects.

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